

Chapter 6

Advances in Analytical Techniques

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1. Raman Spectroscopy

It is a non-destructive analytical technique used to provide extensive information about a material's chemical structure, phase, and polymorphism. It is based on inelastic scattering of light, in which the dispersed light has a different energy than the incident light. The energy difference corresponds to the vibrational modes of the molecules in the sample. Discovery: Chandrasekhara Venkata Raman, in 1928, discovered the Raman scattering phenomenon. Working: Fourier Transform Spectroscopy gives Raman spectra. Rotational and vibrational transitions are observed in Raman spectra, as shown in Figure 1.

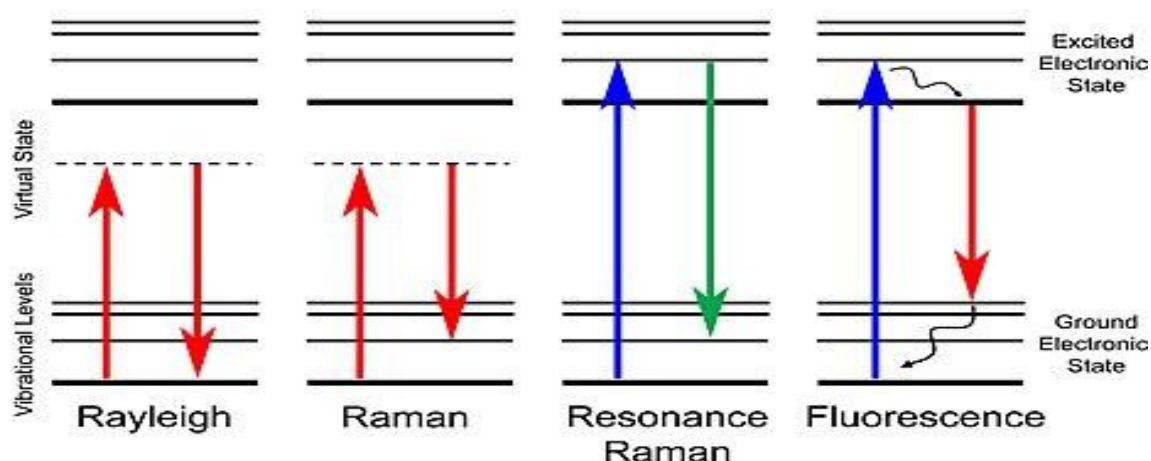


Figure 1. Energy level diagram: Rayleigh scattering, Raman scattering (specifically Stokes scattering), resonance

Figure 2. Raman scattering, and fluorescence

Laser Excitation: A high-intensity laser beam is focused on the sample. **Rayleigh scattering:** The majority of the light is scattered elastically, retaining its original energy and wavelength. This is known as Rayleigh scattering and does not provide relevant information.

Raman Scattering: Inelastic scattering, often known as Raman scattering, occurs in a small amount of light (approximately 0.0000001%). During this process, light interacts with the molecule, causing it to vibrate. The molecule can gain or lose energy, causing a shift in the wavelength of the dispersed light.

Spectral analysis involves collecting and analyzing scattered light using a spectrometer. The resulting spectrum contains peaks representing the vibrational energy levels of the molecules in the sample. These peaks serve as a distinctive “fingerprint” for the substance’s analysis.

Raman Shift: The difference in energy between incident and dispersed light. **Raman Stokes:** When a molecule absorbs energy, the scattered light has a longer wavelength and lower energy than the incident light. **Anti-Stokes Raman** occurs when a molecule loses energy, resulting in scattered light with a shorter wavelength (higher energy) than incident light. **Raman spectrum:** A depiction of Raman scattered light intensity vs. Raman shift.

1.1. Instrumentation of Raman Spectroscopy

Raman Radiation generally includes the following main components, as also shown in Figure 2.

Light source Laser: The most common light source is a laser, which produces a high-intensity monochromatic beam. Common laser types: Nd:YAG, He-Ne, diode lasers. **Choice of wavelength:** The choice of laser wavelength depends on the sample and the desired Raman shift range.

Model illumination and collection optics. **Model Mount:** Holds the model in the correct position. **Focusing optics:** The laser beam is focused on the sample. **Collecting optics** collect Raman scattered light. **Filters** remove highly scattered Rayleigh light to improve the Raman signal.

Monochromatic radiation separates scattered Raman light into its wavelengths. The **plotter** records the intensity of scattered Raman light at each wavelength. Common detectors are Charge-coupled device (CCD), photomultiplier tube (PMT), and GaAs detector.

Data acquisition and processing system: The computer controls the radiation, receives data, and processes the Raman. Software provides tools for data analysis, visual interpretation, and visualization.

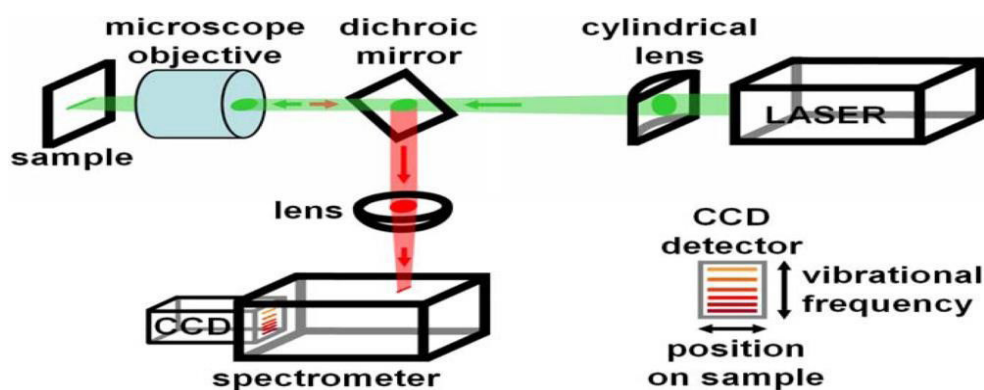


Figure 3. Systematic diagram of Raman Spectroscopy

1.2. Key Concepts in Raman Signaling Instruments

Sensitivity: The ability to detect weak Raman signals is important for the programs. **Spectral resolution:** the ability to distinguish adjacent Raman bands. **Spatial resolution:** the ability to analyze small sample volumes and specific areas of a sample. **Sample compatibility:** The dynamic

measurement must be compatible with the type of sample to be analyzed (solid, liquid, gas). User-friendly software with intuitive functions is essential for everyday analysis.

Advances in Raman spectroscopy instruments:

Advances in Raman spectroscopy have led to the development of:

Portable Raman spectrometers: for in-situ analysis and field applications. Confocal Raman microscopy: for high-resolution spatial imaging. Surface-Enhanced Raman Spectroscopy (SERS): To increase the Raman signals from small analytes. Raman spectroscopy (TERS): for chemical imaging at the nanoscale. By understanding the components and principles of Raman spectroscopy equipment, scientists and researchers can effectively use this technique in a wide variety of applications.

1.3. Key Features

Non-destructive and rapid analysis. Applicable to a wide range of materials, including solids, liquids, and gases. Gives information about the structure and composition of molecules.

1.4. Applications

Raman spectroscopy has a wide range of applications in various fields.

Chemistry: Identifying and characterizing compounds, studying chemical reactions. Materials science: Analyzing materials properties, determining crystal structure, and studying phase transitions. Biology and medicine: Studying biomolecules, diagnosing diseases, and monitoring drug delivery. Geology and mineralogy: Identifying minerals and gemstones, studying geological formations. Food science: Analyzing evidence, identifying materials, and investigating crimes.

1.5. Advantages over Infrared Spectroscopy

While both Raman and Infrared spectroscopy provide information about molecular vibrations, Raman spectroscopy has some advantages.

Raman spectroscopy can be used to analyze aqueous solutions without interference from water, unlike infrared spectroscopy. Different selection rules for Raman and infrared scattering can complement each other in providing information about molecular structure. Raman spectroscopy often requires minimal sample preparation.

1.6. Limitations

Raman scattering is a weak effect, requiring sensitive detectors. Fluorescent samples can interfere with Raman spectra. Spectra can be complex for large molecules, making interpretation challenging.

2. Terahertz Spectroscopy

Terahertz (THz) spectroscopy is a relatively new analytical technique that explores the electromagnetic spectrum between microwaves and infrared radiation. It falls between 3 mm and 30 μm in radiation wavelength, or the frequency range of 100 GHz–10 THz shown in Figure 3. Terahertz radiation is sometimes referred to as T-waves, T-light, T-gap, T-waves, or T-lux. Because there is limited access to technology for producing and detecting radiation, this type of electromagnetic radiation is less well understood. Additionally, because terahertz radiation is typically very sensitive to a wide range of resonances, including those that are vibrational, translational, rotational, torsional, and conformational, it can provide information about molecules that is not possible to obtain with other analytical and imaging methods. Because of these special qualities, they can be used to image, analyze, and identify a wide range of materials.

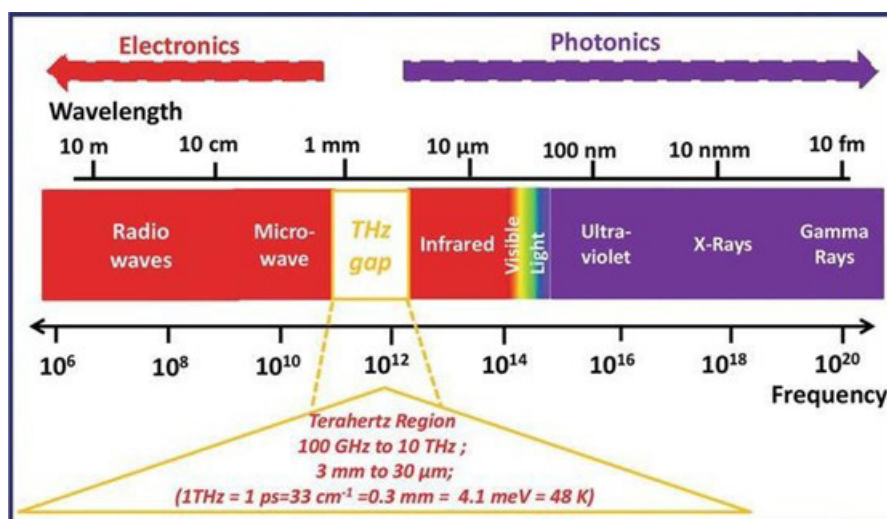


Figure 4. Terahertz Region in wavelengths and frequencies.

Unlike X-rays, THz radiation doesn't ionize matter, making it safe for biological samples. It can penetrate many materials, including paper, plastics, and clothing. Different substances have unique THz signatures, enabling identification and analysis.

2.1. How Terahertz Spectroscopy Works

Most of the time, a terahertz system is made up of a detector, components, and a source. Two or more of these elements may be present in some terahertz systems. Terahertz radiation is produced by a variety of sources, manipulated by elements like mirrors, polarizers, and lenses, and measured by a detector as it approaches it. These include sources pumped by lasers, thermal, vacuum, electronic, solid-state, and mechanically excited terahertz radiation sources. Continuous or pulsed lasers are used in sources that are pumped by lasers. Terahertz radiation is produced by an electro-optic dendrimer using the difference-frequency technique (DFG). The method employs two continuous lasers to provide an electro-optic dendrimer emitter with a combined pump power that can produce a stable terahertz radiation at room temperature, ranging from 0.1 THz to roughly 10 THz. Dendrimers are artificial polymers with a high branching count that consist of an internal structure resembling a tree, an external surface adorned with functional groups, and a central core. Dendrimers are radially symmetric molecules with nanoscale dimensions. A dendrimer's size is determined by its generation, or the quantity of concentric shells surrounding the core; the larger the size, the higher the generation. The optical and electro-optic properties of the functional groups at the dendrimer periphery can be controlled through precise manipulation of the structural features of the dendrimers. Multilayers that self-assemble can be formed by dendrimers. Certain parameters, such as the number of concentric layers or dendrimer generation, can be controlled to alter the thickness of such multilayer assemblies.

Dendrimer thickness is also influenced by solution chemistry and surface characteristics. The process that produces the electro-optic dendrimer is called dendrimer dipole excitation (DDE). Applications that typically depend on nonlinear optical parameters like the electro-optic coefficient (EOC) and the second-order susceptibility, $\chi(2)$, require electro-optic properties. After chromophore doping and poling, a third-generation dendrimer develops into an electro-optic dendrimer with a high electro-optic coefficient and a high second-order susceptibility ($\chi(2)$). For both the bandwidth and the up conversion, a high $\chi(2)$ is necessary. The dendrimer film's dipole alignment is optimized by chromophore poling. Numerous poling techniques exist, such as photo-assisted poling, contact electrode poling, optical poling, and corona poling. As demonstrated in Eq. (1), where E_p is the poling field

$$|\Delta n| = 1/2 n^3 r_{33} E_p \quad (1),$$

the electro-optic coefficient (r_{33}) is derived from the difference in the refractive index (Δn) of poled and unpoled electro-optic dendrimers in accordance with Pockel's law. Dendrimers have a much higher electro-optic coefficient than inorganic crystalline materials because, as Eq. (2) illustrates, second-order susceptibility is proportional to ϵ , the relative electric constant, and r_{33} . Dendrimer doping produces a dipole moment (μ) distribution of charge (Q) carriers in the dendrimer, which modifies the equation for dipole moment ($\mu = ql$) to $\mu(r) = Ql(r)$. This results in

$$\chi^{(2)} \partial \epsilon_2 r_{33} \quad (2),$$

Difference-frequency generation (DFG) is used to generate terahertz radiation from the electrooptic dendrimers by a nonlinear polarization as illustrated in Eq. (3).

$$P(\omega_1 - \omega_2) = 2 \chi^{(2)} E_1 E_2 n \quad (3),$$

where E_1 and E_2 are the field strengths, ω_1 and ω_2 are the frequencies of the input lasers. Every photon created at the difference-frequency (Eq. (4)) requires the destruction of a photon at a higher input frequency (ω_1) and the creation of a photon at the lower input frequency (ω_2) to meet the requirements for energy conservation:

$$\omega_3 = \omega_1 - \omega_2 \quad (4)$$

The radiation is produced by a terahertz source, passes through a number of parts, and ultimately arrives at the detector. Terahertz radiation can be found in a variety of ways. This section will cover the terahertz detection technique of electro-optic sampling. The linear electro-optic effect, sometimes referred to as Pockel's effect, is the foundation of electrooptic sampling. An electro-optic material's birefringence is modulated by an externally applied electric field in the Pockel's effect. An electro-optic medium, a broadband quarter-wave plate, a Wollaston prism, and a differential detector are typical parts of this detection system. When terahertz waves enter the detector, an incident THz pulse causes an electro-optic medium to exhibit birefringence that is proportionate to the pulse's electric field. Varying birefringence is used to measure a probe near-infrared probe pulse's change in polarization state. By determining the degree of polarization rotation as a function of the delay between the near-infrared probe pulse and the terahertz pulse, the terahertz electric field can be mapped.

2.2. Application of Terahertz Spectroscopy

The unique properties of THz radiation make it suitable for a wide range of applications: Material Science: Characterizing materials, studying phase transitions, and analyzing defects. Biomedical Imaging: Non-invasive imaging of biological tissues, cancer detection, and drug delivery studies. Security: Detecting concealed objects, explosives, and contraband. Pharmaceutical Industry: Analyzing drug formulations and polymorphs. Food Safety: Detecting contaminants and adulterants in food products.

2.3. Challenges and Future Directions

While THz spectroscopy offers significant potential, challenges such as generating and detecting THz radiation: It develops efficient and cost-effective sources and detectors.

Data analysis: is processing and interpreting complex THz spectra. Hyper spectral imaging: Capturing the Spectrum

The method known as hyperspectral imaging gathers and analyzes data from all across the electromagnetic spectrum to determine the spectrum of each pixel in an image. This makes it possible to identify materials and objects by examining their distinctive spectral signatures. Combining the ideas of spectroscopy and imaging, hyper spectral imaging is a potent method. It takes pictures at multiple, continuous spectral bands, forming a data cube that provides fine-grained details about the constituents of an image.

Imaging using several bands from the electromagnetic spectrum is known as spectral imaging. While the RGB camera captures images using the three visible light bands (red, green, and blue), hyperspectral imagery allows the analysis of object interactions with many more bands, including thermal infrared and bands ranging from 250 nm to 15,000 nm. Spectroscopy, also known as spectral sensing, is the study of the interaction between light and matter.

Over the past 20 years, the use and prominence of two related technologies—hyperspectral and multispectral imaging—have increased. Although the terms are sometimes used interchangeably, they actually refer to two different imaging techniques, each with specific application areas. Compared to traditional machine vision imaging techniques, which make use of visible light (400-700nm). Both technologies have advantages. These advantages do however, come with a higher level of system complexity with regard to filtering, lighting, and optical design. In typical machine vision applications, illumination used and captured by the sensor is in the visible spectrum. This part of the spectrum consists of the only light that the human eye can detect, ranging from roughly 400nm (violet) to 700nm (dark red) (Figure 6). Imaging lens assemblies and sensors typically have peak spectral sensitivities around 550nm. The quantum efficiency of a camera sensor is the ability to convert photons into an electric signal; this efficiency decreases significantly into the ultraviolet or the near infrared. Hyperspectral imaging (HSI) is, to put it simply, a technique for taking pictures that include data from a wider range of the electromagnetic spectrum. This part of the spectrum may begin in the ultraviolet, move through the visible range, and conclude in the near- or short-wave infrared. This wider range of wavelengths can highlight characteristics of the material composition that are not readily visible.

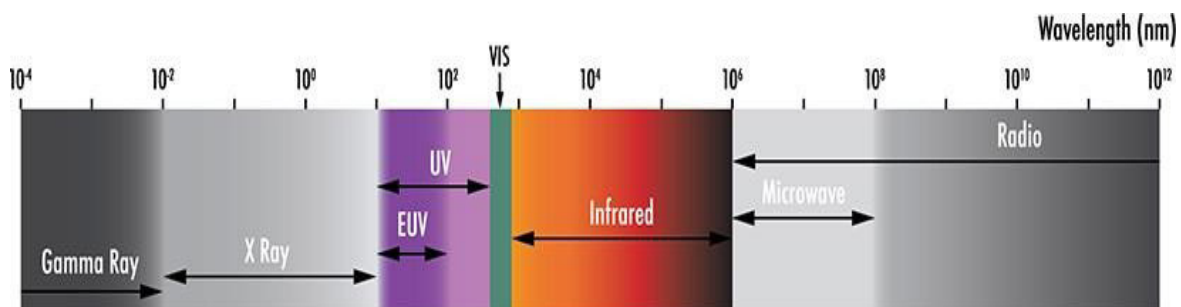


Figure 5. The human eye can only see a small portion of the wavelength spectrum; hyperspectral and multispectral imaging uses wavelength regions that are not in the visible spectrum.

How it Works?

Spectral Acquisition: A hyper spectral sensor captures hundreds of thousands of narrow, contiguous spectral bands for each pixel in an image.

Data Cube Formation: The collected data is organized in to a three-dimensional data cube, where two dimensions represent spatial information (x, y), and the third dimension represents spectral information (wavelength).

Analysis: Specialized software processes the data cube to extract meaningful information about the materials present in the image.

An array of grayscale values produced by machine vision sensors creates a two-dimensional image of the object within the viewing area. Generally speaking, this is useful for feature recognition in order to sort, measure, or locate objects. Unless optical filters are used, the vision system is not aware of the wavelengths being used for illumination. This isn't the case for sensors that use a Bayer Pattern (RGB) filter, but even in those cases, the camera software ultimately determines color because each pixel can only receive light from a specific range of wavelengths. Every pixel in a fully hyperspectral image corresponds to coordinates, wavelength information, and signal intensity. This is the reason why imaging spectroscopy is a common term for HSI. Just to refresh your memory, a spectrometer measures relative intensity for each wavelength it detects in addition to wavelength information. Usually, these instruments gather light from a single source or spot on a sample. Materials that scatter and reflect particular wavelengths, as well as the composition of materials based on emissions of fluorescence or phosphorus, can be found using a spectrometer. By giving positional information to the gathered light, an HSI system advances this technology ranges. A hyperspectral system produces an image cube or hyperspectral data cube rather than a 2D picture.

There are four main modes of hyperspectral acquisition that are used, and each has pros and cons (Figure 7). One spatial coordinate's worth of spectral information is acquired at a time using the point scanning technique known as the whiskbroom method. Although the system must scan the target area on both the x and y axes, this method tends to offer the highest level of spectral resolution, but it also adds a significant amount of time to the acquisition process. Using a row of pixels to scan over an area and capture both spectral and positional information, the pushbroom method is a line scanning data capture technique that requires a single axis of spatial movement. "Compact size, low weight, simpler operation, and higher signal to noise ratio" are possible features of these pushbroom systems. When the process known as "plane scanning" takes multiple picture captures to produce the spectral depth of the hyperspectral data cube, simultaneously imaging the entire 2D region at each wavelength interval. The accuracy of positional and spectral information will be compromised if the subject moves during the acquisition process, even though this capture method does not require translation of the sensor or entire system. Single shot, or snapshot, hyperspectral image acquisition is the fourth and most recent mode of operation. The complete hyperspectral data cube is captured by a single

shot imager in a single integration time. While single shot seems to be the way forward for HSI implementation, its current limitations stem from its relatively lower spatial resolution.

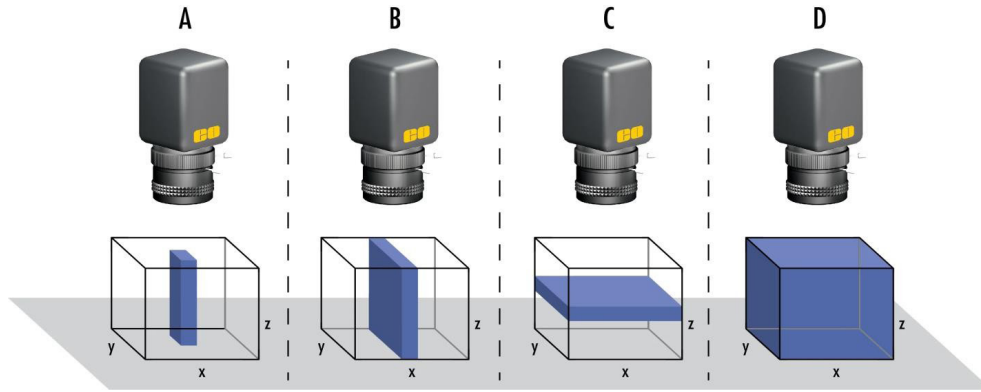


Figure 7. It shows the four main modes of hyperspectral acquisition: single shot mode (D), plane scanning (C), area scanning mode (D), line scanning (B), and point scanning (A), also known as pushbroom or whiskbroom mode.

While hyperspectral and multispectral imaging (MSI) systems are similar, they differ significantly in some important ways. An MSI focuses on a few preselected wavebands depending on the application at hand, as opposed to an HSI's practically continuous wavelength data collection. Common RGB sensors serve as an example of this concept even though they are not a direct comparison or example. Red, green, and blue filters make up the Bayer pattern that is superimposed on RGB sensors. These filters attenuate the remaining light while allowing pixels to absorb wavelengths from particular color bands. The bandpass filters exhibit a slight spectral overlap and transmission bands between 400 and 700 nm. Figure 8 shows an illustration of this. After that, false color is added to the photos to make them resemble what the human eye would see. The wavelength bands used in most multispectral imaging applications are much narrower and more numerous. The wavebands are not limited to the visible spectrum; they are typically on the order of tens of nanometers.

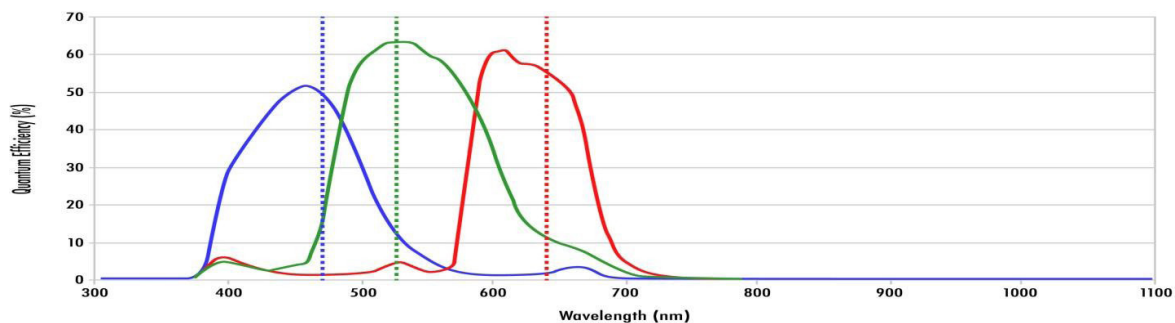


Figure 8. The overlap between red, green, and blue is displayed in the quantum efficiency curve for an RGB camera.

Some consider MSI to be a lower spectral resolution variant of HSI. Actually, each of the two technologies has benefits of its own that make them a better tool for certain jobs.

Applications sensitive to minute variations in signal along a continuous spectrum are best suited for HSI. A system that samples larger wavebands might miss these tiny signals. Nevertheless, in order for certain systems to selectively capture light, a sizable portion of the electromagnetic spectrum must be blocked (Figure 9). Significant noise at the other wavelengths could interfere with measurements and observations. Additionally, the spectral information in the wavelengths (mid-wave IR) can also have isolated channels, depending on the application. Quicker image capture, processing, and analysis are possible when the image is included in the data cube.

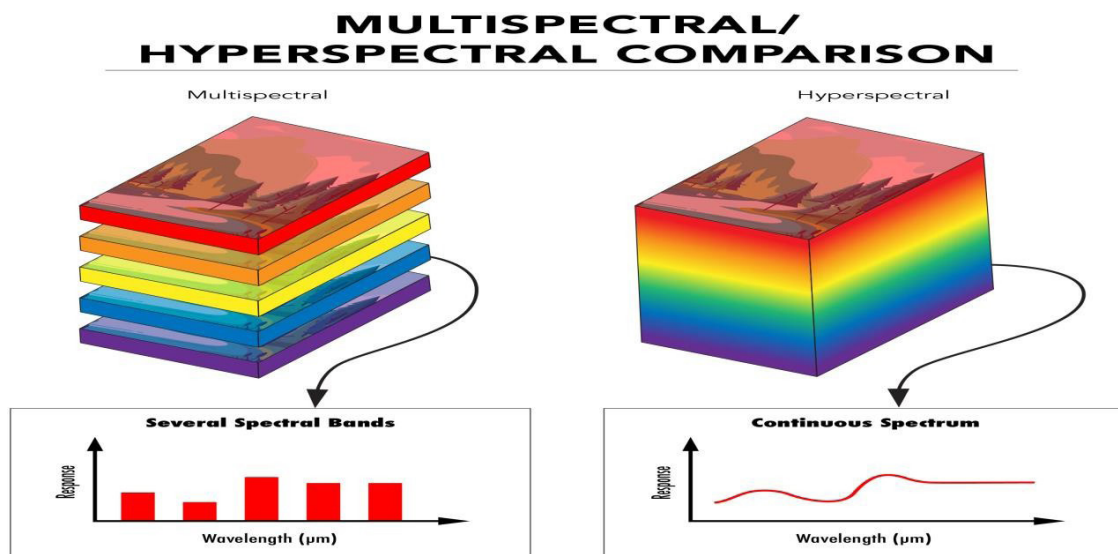


Figure 9. A comparative analysis of the image stacks obtained from hyperspectral imaging (images taken in many different spectra) and multispectral imaging (images taken in several different spectra).

2.4. Applications

The number of application areas where HSI and MSI must be used is constantly increasing. For many years, both HSI and MSI have been essential to remote sensing—the aerial imaging of the earth's surface through the use of satellites and unmanned aerial vehicles (UAVs). Spectral photography provides an unobstructed view of the ground below by penetrating Earth's atmosphere and various cloud layers. With the aid of this technology, population fluctuations, geological changes, and archeological site investigations can all be tracked. Furthermore, the study has shown that HSI and MSI technologies are becoming more and more important. Data can be collected about deforestation, ecosystem degradation, carbon recycling, and increasingly erratic weather patterns. Researchers use the information gathered to create predictive models of the global ecology, which drives many environmental initiatives meant to combat the negative effects of climate change and human influence on nature.

2.5. Geology

Data can be collected about deforestation, ecosystem degradation, carbon recycling, and increasingly erratic weather patterns. Researchers use the information gathered to create predictive models of the global ecology, which drives many environmental initiatives meant to combat the negative effects of climate change and human influence on nature.

In the medical field, this is also true. Thanks to hyperspectral imaging, physicians can now perform non-invasive skin scans to identify diseased or malignant cells. A more thorough understanding of a patient's condition is made possible by certain wavelengths that are better suited for penetrating deeper into the skin. Since cancerous and other diseased cells will fluoresce and absorb light when stimulated appropriately, it is now simple to distinguish them from healthy tissue. It is no longer necessary for medical professionals to speculate based only on what they observe and a patient's description of their symptoms. Advanced technologies have the ability to capture and automatically decipher the spectral data, which greatly accelerates diagnosis and treatment of the precise areas that require attention. These technologies have had a significant impact on a number of fields, including remote sensing and life sciences. Agriculture, pharmaceuticals, food safety and quality, and healthcare are some of the more specialized market sectors. Because these tools let farmers track the growth of their crops, they are especially helpful to farmers. Using spectral imagers, tractors and drones can conduct lower altitude remote sensing by scanning across fields. After that, the farmers examine the captured images' spectral properties. Environmental Monitoring: Assessing water quality, monitoring pollution, and studying vegetation health. Food Inspection: Detecting contaminants, verifying food authenticity, and monitoring quality. Defense and Security: Target detection, surveillance, and material identification.

2.6. Key advantages

Detailed Spectral Information: Provides a comprehensive spectral signature for each pixel, enabling precise material identification. Spatial Context: Combines spectral information with spatial location, allowing for mapping and analysis of material distribution. Non-destructive: Does not require physical contact or sample preparation. Versatility: Applicable to a wide range of applications across different industries.

2.7. Challenges

Hyperspectral images generate massive amounts of data, requiring efficient processing and storage. Extracting meaningful information from hyperspectral data often requires advanced algorithms and expertise. Hyperspectral imaging systems can be expensive, limiting their widespread adoption.

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